

## CLAIMS:

1. A method of making a genetically modified plant comprising:
  - (a) introducing into a plant cell capable of being transformed and regenerated to a whole plant:
    - (i) a DNA construct comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,
    - (ii) a DNA construct comprising a repressor gene whose gene product is capable of repressing the activity of said lethal gene product;
  - (b) regenerating from said plant cell a whole plant; and,
  - (c) selecting a plant wherein the inserted repressor gene segregates independently from the inserted repressible lethal gene.
2. The method of claim 1 wherein the DNA construct comprising the repressible lethal gene is separate from the DNA construct comprising the repressor gene, and wherein selection is accomplished by crossing the plants generated in step (b)..
3. The method of claim 1 wherein the inserted repressible lethal gene and the inserted repressor gene are

located on opposite sister chromosomes of a chromosome pair.

4. The method of claim 1 wherein the DNA construct comprising the repressible lethal gene is the same as the DNA construct comprising the repressor gene, and wherein independent segregation is effected by use of a site-specific recombinase or a transposase.

5. The method of claim 1 wherein the repressible lethal gene or the repressor gene or both is under control of a tissue-specific promoter.

6. The method of claim 1 wherein the DNA construct comprising the repressible lethal gene also comprises a specific DNA operator sequence, wherein the repressor is a bacterial repressor capable of binding to the specific DNA operator sequence, and wherein the binding between the bacterial repressor and the specific DNA operator sequence results in repression of the lethal gene.

7. The method of claim 1 wherein the repressor gene is under control of a constitutive promoter.

8. The method of claim 1 wherein the repressor gene is under control of an inducible promoter.

9. The method of claim 1 wherein the activity of the lethal gene product comprises over-expression or under-expression of a naturally occurring plant growth regulating substance.

10. The method of claim 1 wherein the repressible lethal gene is selected from the group consisting of: oncogenes 1 and 2, oncogene 4 of *Agrobacterium*, a gene encoding a ribosome inactivating protein, a gene encoding diphtheria A chain toxin, and a gene encoding a ribonuclease. X

11. The method of claim 1 wherein the DNA construct comprising the repressible lethal gene further comprises a conditionally lethal gene.

12. The method of claim 1 wherein the DNA construct comprising the repressible lethal gene is separate from the DNA construct comprising the repressor gene, and wherein the constructs are introduced simultaneously into a plant cell capable of being transformed and regenerated into a whole plant.

13. The method of claim 1 wherein the DNA construct comprising the repressible lethal gene is the same as the DNA construct comprising the repressor gene, said DNA

construct further comprising a DNA sequence that allows independent segregation of the inserted repressible lethal gene and the inserted repressor gene.

14. A method of making a genetically modified plant comprising:

(a) introducing into a plant cell capable of being transformed and regenerated to a whole plant:

(i) a DNA construct comprising a first repressible lethal gene, the activity of whose gene product is lethal to plant cells;

(ii) a DNA construct comprising a first repressor gene whose gene product is capable of repressing said first lethal gene activity;

(iii) a DNA construct comprising a second repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,

(iv) a DNA construct comprising a second repressor gene whose gene product is capable of repressing said second lethal gene activity;

(b) regenerating from said plant cell a whole plant; and,

(c) selecting a plant wherein the inserted first repressor gene segregates independently from the inserted first repressible gene, and wherein the inserted second repressor gene segregates independently from the inserted second repressible gene.

15. The method of claim 14 wherein the DNA construct comprising the first repressible lethal gene is separate from the DNA construct comprising the first repressor gene; wherein the DNA construct comprising the second repressible lethal gene is separate from the DNA construct comprising the second repressor gene; and wherein selection is accomplished by crossing the plants generated in step (b).

16. The method of claim 14 wherein the inserted first repressible lethal gene and the inserted first repressor gene are located on opposite sister chromosomes of a chromosome pair, and wherein the inserted second repressible lethal gene and the inserted second repressor gene are located on opposite sister chromosomes of a chromosome pair.

17. The method of claim 14 wherein the first repressible lethal gene is within the same DNA construct as the first repressor gene, and wherein the second repressible lethal gene is within the same DNA construct as the second repressor gene; and wherein independent segregation is effected by use of a site-specific recombinase or a transposase.

18. The method of claim 14 wherein the first repressible lethal gene is linked to the second repressor

gene, and wherein the second repressible lethal gene is linked to the first repressor gene.

19. The method of claim 14 wherein the first or second or both repressible lethal gene is under control of a tissue-specific promoter.

20. The method of claim 14 wherein:

(a) the DNA construct comprising the second repressible lethal gene also comprises a first specific DNA operator sequence, wherein the first repressor is a bacterial repressor capable of binding to the first specific DNA operator sequence, and wherein the binding between the first bacterial repressor and the first specific DNA operator sequence results in repression of the second lethal gene; and,

(b) the DNA construct comprising the first repressible lethal gene also comprises a second specific DNA operator sequence, wherein the second repressor is a bacterial repressor capable of binding to the second specific DNA operator sequence, and wherein the binding between the second bacterial repressor and the second specific DNA operator sequence results in repression of the first lethal gene.

21. The method of claim 14 wherein the first or second or both repressor gene is under control of a tissue-specific promoter.

22. The method of claim 14 wherein the first, or first and second, repressor gene is under control of a constitutive promoter.

23. The method of claim 1 or 14 wherein the repressor comprises an antisense RNA or a sense gene capable of inhibiting expression of the repressible lethal gene.

24. The method of claim 1 or 14 wherein the repressor comprises a ribozyme capable of inhibiting expression of the repressible lethal gene.

25. The method of claim 14 wherein either or both repressible lethal genes is selected from the group consisting of: oncogenes 1 and 2 of *Agrobacterium*, oncogenes 1 and 2 of *Pseudomonas*, oncogene 4 of *Agrobacterium*, a gene encoding a ribosome inactivating protein, a gene encoding diphtheria A chain toxin, and a gene encoding a ribonuclease.

26. The method of claim 10 or 25 wherein oncogenes 1 and 2 are from *Agrobacterium* or *Pseudomonas*.

~~27. The method of claim 10 or 25 wherein oncogene 4 is from *Agrobacterium*.~~

- 27 ~~28~~. The method of claim 10 or 25 wherein the ribonuclease is Barnase or T1 ribonuclease.
- 28 ~~29~~. The method of claim 14 wherein the DNA construct comprising a first repressible lethal gene, or the DNA construct comprising a second repressible lethal gene, or both, further comprises a conditionally lethal gene.
- 29 ~~30~~. The method of claim 14 wherein the DNA construct comprising the first repressible lethal gene is separate from the DNA construct comprising the first repressor gene; wherein the DNA construct comprising the second repressible lethal gene is separate from the DNA construct comprising the second repressor gene, and wherein the constructs are introduced simultaneously into a plant cell capable of being transformed and regenerated into a whole plant.
- 30 ~~31~~. The method of claim 14 wherein the DNA construct comprising the first repressible lethal gene is the same as the DNA construct comprising the first repressor gene; wherein the DNA construct comprising the second repressible lethal gene is the same as the DNA construct comprising the second repressor gene, said DNA constructs further comprising a DNA sequence that allows independent segregation of the inserted first repressible lethal gene from the inserted first repressor gene, and that allows



independent segregation of the inserted second repressible lethal gene from the inserted second repressor gene.

- 31 ~~32~~. The method of claim 14 wherein the DNA construct comprising the first repressible lethal gene is the same as the DNA construct comprising the second repressor gene; wherein the DNA construct comprising the second repressible lethal gene is the same as the DNA construct comprising the first repressor gene, said DNA constructs being contained within a single plant transformation vector that allows independent segregation of the DNA constructs.

- 32 ~~33~~. The method of claim <sup>31</sup>~~32~~ wherein independent segregation is effected by use of a site-specific recombinase or a transposase.

- 33 ~~34~~. The method of any one of claims 5, 19 and 21 wherein the tissue specific promoter is a seed specific promoter.

- 34 ~~35~~. The method of claim <sup>33</sup>~~34~~ wherein the seed specific promoter is the phaseolin promoter.

- 35 ~~36~~. The method of claim 1 or 14 wherein the plant further comprises a novel trait.

36 ~~37~~. The method of claim ~~36~~<sup>35</sup>, wherein the novel trait comprises an altered phenotype, a protein not found in the native plant cell, or a protein not found in the native plant cell and which confers no detectable phenotype.

37 ~~38~~. The method of claim ~~36~~<sup>35</sup> or ~~37~~<sup>36</sup>, wherein the gene encoding the novel trait is linked to a repressible lethal gene.

38 ~~39~~. The method of any one of claims ~~36~~<sup>35</sup> to ~~38~~<sup>37</sup>, wherein the gene encoding the novel trait is under the control of a seed specific promoter.

39 ~~40~~. A method of producing, for a hybrid cross, a male parent comprising a repressible lethal gene that is expressed during outcrossing or introgression of alien germplasm, comprising:

(a) introducing into a plant cell capable of being transformed and regenerated to a whole plant:

(i) a DNA construct comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,

(ii) a DNA construct comprising a repressor gene, whose gene product is capable of repressing said lethal gene activity, under control of an inducible promoter;

wherein the inserted repressor gene segregates independently from the inserted repressible gene;

- (b) regenerating from said plant cell a whole plant;
- (c) generating from said plant, a plant which is homozygous for the repressible lethal gene and inducible repressor gene;

wherein the homozygous plant can function as the male parent for a hybrid cross.

40 ~~41~~. A method of maintaining the male parent of claim ~~40~~, comprising:

- ~~39~~ (a) permitting the homozygous plant to self-pollinate;
- (b) inducing the inducible promoter in the homozygous plant during the period of time that seed formation takes place; and
- (c) obtaining the seed.

41 ~~42~~. A plant produced by the method of claim ~~40~~ or ~~41~~. <sup>39</sup> <sup>40</sup>

42 ~~43~~. A method of producing, for a hybrid cross, a female parent that carries a repressible lethal gene, comprising:

- (a) introducing into a plant cell capable of being transformed and regenerated to a whole plant a DNA construct comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,
- (b) regenerating from said plant cell a whole plant.

43 ~~44~~. A method of producing, for a hybrid cross, a female parent comprising a repressible lethal gene and an inducible repressor gene, comprising:

- (a) introducing into a plant cell capable of being transformed and regenerated to a whole plant:
  - (i) a DNA construct comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,
  - (ii) a DNA construct comprising a repressor gene, whose gene product is capable of repressing said lethal gene activity, under control of an inducible promoter;

wherein the inserted repressor gene segregates independently from the inserted repressible gene;

- (b) regenerating from said plant cell a whole plant;
- (c) generating from said plant, a plant which is homozygous for the repressible lethal gene and inducible repressor gene;

wherein the homozygous plant can function as the female parent for a hybrid cross.

44 45. A method of maintaining the female parent of claim ~~44~~, comprising:

43  
(a) permitting the homozygous plant to self-pollinate;

(b) inducing the inducible promoter in the homozygous plant during the period of time that seed formation takes place; and

(c) obtaining the seed.

45 46. A plant produced by the method of any one of claims ~~43~~ to ~~45~~.

42 44

39 40 42 44  
46 47. The method of any one of claims ~~40~~, ~~41~~, ~~43~~ to ~~45~~ wherein said seed specific promoter comprises the phaseolin promoter.

39 40 42 44  
47 48. The method of any one of claims ~~40~~, ~~41~~, ~~43~~ to ~~45~~ wherein the plant further comprises a novel trait.

46. ~~49~~. The method of claim <sup>47</sup>~~48~~, wherein the novel trait comprises an altered phenotype, a protein not found in the native plant cell, or a protein not found in the native plant cell and which confers no detectable phenotype.

49 ~~50~~. The method of claim <sup>47</sup>~~48~~ or <sup>48</sup>~~49~~, wherein the gene encoding the novel trait is linked to a repressible lethal gene.

50 ~~51~~. The method of any one of claims <sup>47</sup>~~48~~ to <sup>49</sup>~~50~~, wherein the gene encoding the novel trait is under the control of a seed specific promoter.

51 ~~52~~. A method of producing hybrid seed comprising a repressible lethal gene and a repressor gene, comprising:

- (a) allowing the plant of claim <sup>45</sup>~~48~~ to flower;
- (b) fertilizing said plant with pollen which comprises a repressor gene whose gene product is capable of repressing said repressible lethal gene; and,
- (c) recovering hybrid seed which comprises a repressible lethal gene and a repressor gene.

52 ~~53~~. A method of producing, for a hybrid cross, a female parent that carries a repressor gene, comprising:

- (a) introducing into a plant cell capable of being transformed and regenerated to a whole plant a DNA construct comprising a repressor gene;
- (b) regenerating from said plant cell a whole plant;
- (c) generating from said plant a plant which is homozygous for the repressor gene;

wherein the homozygous plant can function as the female parent for a hybrid cross.

<sup>52</sup>  
~~53~~ ~~54~~. The method of claim ~~53~~ wherein the female parent is incapable of self-pollination.

<sup>52</sup> <sup>53</sup>  
~~54~~ ~~55~~. A plant produced by the method of claim ~~53~~ or ~~54~~.

~~55~~ ~~56~~. A method of making a genetically modified plant seed comprising a repressible lethal gene that is expressed during outcrossing or introgression of alien germplasm, comprising:

- (a) crossing the <sup>54</sup>male parent of claim <sup>41</sup>~~42~~ with the female parent of claim ~~55~~, wherein the repressor gene product of the female parent is capable of repressing the repressible lethal gene of the male parent;

(b) harvesting hybrid seed from the male parent, comprising the repressible lethal gene from the male parent and the repressor gene from the female parent.

56 <sup>55</sup> 57. The method of claim ~~56~~ wherein the plant seed is self-incompatible.

*derived from a self-incompatible plant species.*

X

57 <sup>38 51 55 56</sup> 58. A plant produced by the method of any one of claims 1 to ~~39, 52, 56~~ and ~~57~~.

58 <sup>41 45 54 57</sup> 59. The plant of any one of claims ~~42, 46, 55~~ and ~~58~~, further comprising a marker gene.

59 <sup>57 58</sup> 60. The plant of claim ~~58~~ or ~~59~~, which is a dicot (Dicotyledoneae).

60 <sup>57 58</sup> 61. The plant of claim ~~58~~ or ~~59~~, which is a monocot (Monocotyledoneae).

61 <sup>57 58</sup> 62. The plant of claim ~~58~~ or ~~59~~, which is a member of the family: Malvaceae, Linaceae, Compositae, Fabaceae, Euphorbiaceae, or Oleaceae.



62 ~~63~~. The plant of claim ~~58~~<sup>57</sup> or ~~59~~<sup>58</sup>, which is a member of the genus: *Brassica*.

63 ~~64~~. The plant of claim ~~58~~<sup>57</sup> or ~~59~~<sup>58</sup>, which is a member of the genus: *Linum*, *Gossypium*, *Glycine*, *Arachis*, *Carthamus*, *Helianthus*, *Sinapis*, *Raphanus*, *Ricinus* or *Olea*.

64 ~~65~~. The plant of claim ~~58~~<sup>57</sup> or ~~59~~<sup>58</sup>, which is a member of the family *Brassicaceae* (= *Cruciferae*).

65 ~~66~~. The plant of claim ~~58~~<sup>57</sup> or ~~59~~<sup>58</sup>, which is a *Brassica napus* or *rapa*.

66 ~~67~~. The plant of claim ~~58~~<sup>57</sup> or ~~59~~<sup>58</sup>, which is a rapeseed or canola.

67 ~~68~~. The plant of claim ~~58~~<sup>57</sup> or ~~59~~<sup>58</sup>, which is an oilseed plant.

68 ~~69~~. A plant transformation vector, for use in limiting outcrossing of a plant transformed with said vector, comprising:

(a) a repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,

(b) a repressor gene whose gene product is capable of repressing the activity of said lethal gene product;  
*said repressible lethal gene and said repressor gene being located so that they segregate independently.* X

69 70. A plant transformation vector, for use in limiting introgression of alien germplasm, comprising:

(a) a repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,

(b) a repressor gene whose gene product is capable of repressing the activity of said lethal gene product;  
*said repressible lethal gene and said repressor gene being located so that they segregate independently.* X

70 71. A plant transformation vector, for use in limiting outcrossing of a plant transformed with said vector or for limiting introgression of alien germplasm, comprising:

(a) a first repressible lethal gene, the activity of whose gene product is lethal to plant cells;

(b) a first repressor gene whose gene product is capable of repressing said first lethal gene activity;

(c) a second repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,

(d) a second repressor gene whose gene product is capable of repressing said second lethal gene activity.

<sup>70</sup>  
71 ~~72~~. The vector of claim ~~71~~<sup>70</sup>, wherein the first repressible lethal gene is not linked to the first repressor gene, and wherein the second repressible lethal gene is not linked to the second repressor gene.

<sup>70</sup>  
72 ~~73~~. The vector of claim ~~71~~<sup>70</sup>, wherein the first repressible lethal gene is linked to the first repressor gene, and wherein the second repressible lethal gene is linked to the second repressor gene, and wherein the vector further comprises at least one DNA sequence that allows independent segregation of the first repressible lethal gene from the first repressor gene, and independent segregation of the second repressible lethal gene from the second repressor gene, after transformation.

<sup>70</sup> <sup>72</sup>  
73 ~~74~~. The vector of any one of claims ~~71~~<sup>70</sup> to ~~73~~<sup>72</sup>, wherein:

(a) a first specific DNA operator sequence is linked to the second repressible lethal gene, wherein the first repressor is a bacterial repressor capable of binding to the first specific DNA operator sequence, and wherein the binding between the first bacterial repressor and the first specific DNA operator sequence results in repression of the second lethal gene; and,

(b) a second specific DNA operator sequence is linked to the first repressible lethal gene, wherein the second repressor is a second bacterial repressor capable of binding to the second specific DNA operator sequence, and wherein the binding between the second bacterial repressor

and the second specific DNA operator sequence results in repression of the first lethal gene.

74 75. A plant transformation vector, for use in generating a parent plant for a hybrid cross, comprising:

(a) a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,

(b) a repressor gene, whose gene product is capable of repressing said lethal gene activity, under control of an inducible promoter.

75 76. The vector of any one of claims <sup>68 69 74</sup>~~68~~, ~~69~~ and ~~75~~, wherein the repressible lethal gene and the repressor gene are not linked.

76 77. The vector of any one of claims <sup>68 69 74</sup>~~68~~, ~~69~~ and ~~75~~, wherein the repressible lethal gene and the repressor gene are linked, and wherein the vector further comprises at least one DNA sequence that allows independent segregation of the repressible lethal gene and the repressor gene after transformation.

77 78. The vector of claim <sup>72 76</sup> 73 or 77, wherein the at least one DNA sequence comprises a recognition sequence for a site-specific recombinase or a transposase.

78 79. The vector of any one of claims <sup>68 77</sup> 69 to 78, wherein the repressible lethal gene or the repressor gene or both is under control of a tissue-specific promoter.

79 80. The vector of claim <sup>78</sup> 79 wherein the tissue-specific promoter is a seed specific promoter.

80 81. The vector of claim <sup>79</sup> 80 wherein the seed specific promoter is the phaseolin promoter.

81 82. The vector of any one of claims <sup>68 69 74</sup> 69, 70 and 75 to <sup>77</sup> 78, wherein a specific DNA operator sequence is linked to the repressible lethal gene, wherein the repressor is a bacterial repressor capable of binding to the specific DNA operator sequence, and wherein the binding between the bacterial repressor and the specific DNA operator sequence results in repression of the lethal gene.

82 83. The vector of any one of claims <sup>68 77</sup> 69 to 78, wherein the repressor gene is under control of a constitutive promoter.

83 ~~84~~. The vector of any one of claims <sup>68</sup>~~69~~ to <sup>77</sup>~~78~~, wherein the repressor gene is under control of an inducible promoter.

84 ~~85~~. The vector of any one of claims <sup>68</sup>~~69~~ to <sup>77</sup>~~78~~, wherein the activity of the lethal gene product comprises over-expression or under-expression of a naturally occurring plant growth regulating substance.

85 ~~86~~. The vector of any one of claims <sup>68</sup>~~69~~ to <sup>77</sup>~~78~~, wherein the repressible lethal gene is selected from the group consisting of: oncogenes 1 and 2, oncogene 4 of *Agrobacterium*, gene encoding a ribosome inactivating protein, gene encoding diptheria A chain toxin, and a gene encoding a ribonuclease. X

86 ~~87~~. The vector of claim <sup>85</sup>~~86~~ wherein oncogenes 1 and 2 are from *Agrobacterium* or *Pseudomonas*.

~~88. The vector of claim 86 wherein oncogene 4 is from *Agrobacterium*.~~

87 ~~89~~. The vector of claim <sup>85</sup>~~86~~ wherein the ribonuclease is Barnase or T1 ribonuclease.

- 88 ~~90~~. The vector of any one of claims <sup>68</sup>~~69~~ to <sup>77</sup>~~78~~, further comprising a conditionally lethal gene linked to the repressible lethal gene.
- 89 ~~91~~. The vector of any one of claims <sup>68</sup>~~69~~ to <sup>77</sup>~~78~~, wherein the repressor gene is under control of a constitutive promoter.
- 90 ~~92~~. The vector of any one of claims <sup>68</sup>~~69~~ to <sup>77</sup>~~78~~, wherein the product of the repressor gene comprises an antisense RNA or a sense gene or a ribozyme capable of inhibiting expression of the repressible lethal gene.
- 91 ~~93~~. The vector of any one of claims <sup>68</sup>~~69~~ to <sup>77</sup>~~78~~, further comprising a gene encoding a novel trait.
- 92 ~~94~~. The vector of claim <sup>91</sup>~~93~~, wherein the novel trait comprises an altered phenotype, a protein not found in the native plant cell, or a protein not found in the native plant cell and which confers no detectable phenotype.
- 93 ~~95~~. The vector of claim <sup>91</sup>~~93~~ or <sup>92</sup>~~94~~, wherein the gene encoding the novel trait is linked to the repressible lethal gene.

94 ~~96~~. The vector of any one of claims ~~93~~<sup>91</sup> to ~~95~~<sup>93</sup>, wherein the gene encoding the novel trait is under the control of a seed specific promoter.

95 ~~97~~. A plant transformation vector, for use in generating a parent plant for a hybrid cross, comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter.

96 ~~98~~. A plant transformation vector, for use in generating a parent plant for a hybrid cross, comprising a repressor gene.

97 ~~99~~. Plasmid pG1, pG14, pPHAStet1, pGG-14.